appearance when prepared with different fixatives, or even when prepared with the same fixative using different techniques to apply it. Further compounding the problem is that the process by which fixatives worked – for example, what substances in the cell they created bonds with – was generally unknown.[31] This made it difficult to determine whether what was seen in a fixed cell was a structure that was originally in the cell or an artifact.

Although some scientists rejected all use of fixatives, most were convinced that they could in fact reveal real structure in living tissue. Lacking a theoretical understanding of how various fixatives worked, however, these investigators typically had to assess fixatives by their results.[32] But what should the results look like? In light microscopy, investigators could sometimes compare the image of the fixed cell with that of living cells. Observation of the same structures in the unfixed cell provided evidence that the fixative was producing reliable results. Yet, the interest in fixation and staining was to observe structures that could not be seen in living cells. In such a case, researchers had to judge whether the image was likely to correspond to preexisting structures without independent access to that structure. Often, they relied on the plausibility of the image produced. Porter and Kallman (1953), for example, set out some criteria for judging from a micrograph whether the cell had been well fixed:

> Probably the optimum requirements to be made are that the cell should not detectably change its shape under the action of the fixative and that its cytoplasm should not, under high resolution, show discontinuities and lacunae of irregular size and angular form. Such structures with associated surfaces, and density differences, if present in the ground substance of the living cell,

[31] Porter commented on the lack of understanding of how osmium tetroxide functions despite previous studies: "Such studies as those of Hardy (1899), Monckeberg and Bethe (1899), Fischer (1899), Mann (1902), Hofmann (1912), Berg (1927), and Baker (1945) have provided only round indications of the reactions of various fixatives, including osmium tetroxide with proteins and fats" (Porter & Kallman, 1953, p. 127). Six years later Isidore Gersh commented, "It is an impressive commentary on our ignorance of the mechanism of fixation even now, that we do not know why osmium solutions should result in a homogeneous-appearing nucleus, while other fixatives like formol-Zenker result in nuclei with crisp chromatin clumps, or why Bouin's fixative preserves mitochondria only rarely, while potassium dichromate seldom fails to preserve them" (1959, p. 34). Gersh provided the following example of what was known about the operation of osmium: "Osmium oxidizes aliphatic and aromatic double bonds and sulfhydryl groups, alcoholic hydroxyl groups, and some amines. It also has an affinity for certain nitrogenous groups. Sites of oxidation of adjacent hydroxyl or ethylenic groups of adjacent molecules are thought to be bridged by reduced osmium" (1959, p. 38).

[32] Baker comments, "Nowhere in cytological technique has empiricism run riot so freely as in the invention of fixative mixtures" (1942, p. 4).